

GNPDA1 siRNA (h): sc-91867

BACKGROUND

During fertilization in mammals, the sperm activates the egg by causing an increase in the level of free cytoplasmic calcium concentration. This increased calcium concentration induces a characteristic series of oscillations that trigger egg activation and early embryo development. A hamster protein named oscillin is thought to be involved in this pathway. The enzyme glucosamine-6-phosphate isomerase (GNPI) or deaminase (GNPDA1) and the related protein GNPDA2 are the human homologs of hamster oscillin. GNPDA1 and GNPDA2 catalyze the conversion of GNP to fructose-6-phosphate and ammonia. Both proteins exist as homohexamers and are ubiquitously expressed with highest expression in testes, ovary and heart. Three isoforms of GNPDA2 are expressed due to alternative splicing events.

REFERENCES

1. Parrington, J., et al. 1996. Calcium oscillations in mammalian eggs triggered by a soluble sperm protein. *Nature* 379: 364-368.
2. Parrington, J., et al. 1998. A novel protein for Ca^{2+} signaling at fertilization. *Curr. Top. Dev. Biol.* 39: 215-243.
3. Wolosker, H., et al. 1998. Molecularly cloned mammalian glucosamine-6-phosphate deaminase localizes to transporting epithelium and lacks oscillin activity. *FASEB J.* 12: 91-99.
4. Shevchenko, V., et al. 1998. The human glucosamine-6-phosphate deaminase gene: cDNA cloning and expression, genomic organization and chromosomal localization. *Gene* 216: 31-38.
5. Montag, M., et al. 1999. Characterization of testicular mouse glucosamine 6-phosphate deaminase (GNPDA). *FEBS Lett.* 458: 141-144.
6. Amireault, P. and Dube, F. 2000. Cloning, sequencing, and expression analysis of mouse glucosamine-6-phosphate deaminase (GNPDA/oscillin). *Mol. Reprod. Dev.* 56: 424-435.
7. Zhang, J., et al. 2003. Cloning and functional characterization of GNPI2, a novel human homolog of glucosamine-6-phosphate isomerase/oscillin. *J. Cell. Biochem.* 88: 932-940.

CHROMOSOMAL LOCATION

Genetic locus: GNPDA1 (human) mapping to 5q31.3.

PRODUCT

GNPDA1 siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GNPDA1 shRNA Plasmid (h): sc-91867-SH and GNPDA1 shRNA (h) Lentiviral Particles: sc-91867-V as alternate gene silencing products.

For independent verification of GNPDA1 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-91867A, sc-91867B and sc-91867C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNases and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

GNPDA1 siRNA (h) is recommended for the inhibition of GNPDA1 expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

GNPDA1 (237CT2.5.2): sc-517332 is recommended as a control antibody for monitoring of GNPDA1 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor GNPDA1 gene expression knockdown using RT-PCR Primer: GNPDA1 (h)-PR: sc-91867-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.